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Licofelone-A Novel Analgesic and Anti-Inflammatory Agent

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Abstract: Dual inhibitors that block both cyclooxygenase (COX) and lipoxygenase (LOX) metabolic pathways of arachidonic acid are expected to possess clinical advantages over the selective inhibitors of COX enzyme. One of the most promising compounds belonging to this category is licofelone ([2,2 –dimethyl –6-(4-chloropheny-7-phenyl-2,3-dihydro-1H-pyrrazoline-5-yl] acetic acid). Originally discovered by Merckle GmbH and developed by EuroAllaince, licofelone (IC_{50} COX=0.21 μM, IC_{50} 5-LOX=0.18μM) possesses significant analgesic, anti-inflammatory, and antiasthmatic effects at doses that cause no gastrointestinal (GI) side effects. The pharmacodynamic profile of licofelone has been assessed and compared with widely used NSAIDs in different animal models. The ED_{50} value of licofelone is reported to be 11.22-27.07 mg/kg, po and 39.5-55-8 mg/kg, po against carrageenan-induced paw oedema and Randal Selitto hyperalgesic assay in rats, respectively. Licofelone showed analgesic effect (ED_{50} = 31.33 mg/kg) against acetic acid-induced writhing in mice. Licofelone has long duration of action and more effective than indomethacin and zileuton with ED_{50} values of 2.92 mg/kg, po and 36.77 mg/kg, po, in the mechanical hyperalgesia and cold allodynia testing, respectively, against rat model of incisional pain. Licofelone significantly ameliorated indomethacin-induced gastric ulceration, neutrophil adhesion in mesentery, and lipid peroxides in rat gastric mucosa. Also, licofelone reversed the altered vascular permeability, morphological changes, and prevented NSAIDs-related increase in leukotriene levels in gastric mucosa.

The preclinical studies have shown that licofelone not only has convincing pharmacodynamic effect but also it is well tolerated. It is currently under clinical evaluation in osteoarthritis (OA), the most common form of arthritis. The present review describes pharmacological and clinical development of licofelone as a dual inhibitor.

INTRODUCTION

Inflammation is a complex process occurring through a variety of mechanisms, leading to release of various mediators. The mediator arising from the .cyclooxygenase (COX) cascade and the role of biologically active prostaglandins (PGs) in the inflammatory process and body homeostasis have been extensively studied over the years. The outcome of this has been the basis of the use of nonsteroidal anti-inflammatory drugs (NSAIDs) [1]. Three decades ago, Late Sir John Vane and his colleagues established that aspirin and other NSAIDs acted by inhibiting the enzyme cyclooxygenase (COX) which converted arachidonic acid (AA) to prostaglandins (PGs). In 1990s, the breakthrough discovery that cyclooxygenase exists in two different isoforms termed as COX-1, and COX-2, attracted a renewed interest to understand the role of two isoforms in the regulation of prostaglandins synthesis and the biological action under normal and pathophysiological conditions [3]. Recently, a third isoform; COX-3 has been discovered with its skeptic expression in humans [4]. Subsequently, COX hypothesis was proposed, which suggested that COX-2 isoform was induced mainly during inflammation and tradional NSAIDs inhibited it. Whereas COX-1 (constitutive) has housekeeping properties of maintaining gastric mucosa, kidney functions, etc. Soon, it was proposed that the undesirable gastrointestinal (GI) side effects seen with NSAIDs are due to COX-1 inhibition, while the beneficial effects (anti-inflammatory and analgesic) are related to COX-2 inhibition [5]. This gave rise to the discovery of COX-2 inhibitors, a new class of NSAIDs known as coxibs; rofecoxib, celecoxib, etoricoxib, parecoxib, etc., [3]. Coxibs, because of their gastroprotecting properties became the most widely used medications world wide until the recent reports of higher incidence of cardiovascular complications in select group of long-term coxib users led to their withdrawal and/or careful use [6]. With the result there has been a great set back to the wide clinical use of selective COX-2 inhibitors and the need for better and safer therapy for the management of pain and inflammation related chronic disorders.

AA is also a substrate for another enzyme namely 5-lipoxygenase (5-LOX). The 5-LOX pathway is involved in the generation of leukotrienes (LTs) Fig. (**1**). The LTs thus formed are responsible for vascular permeability changes occurring during acute inflammation and play an important role in NSAIDs-induced gastrointestinal damage. Interestingly, neither non selective nor selective COX-2 inhibitors inhibit this enzyme. The inhibition of COX enzyme may lead to a shunt of AA metabolism towards 5-LOX pathway, and therefore, treatment with NSAIDs increase the formation of LTs possibly leading to gastric damage [7]. Co-medication of 5-LOX inhibitors or leukotriene receptor antagonists with NSAIDs has been shown to be beneficial not only in relieving pain and inflammation but also in preventing NSAIDs-induced gastric damage. Thus, the concept of dual inhibition i.e. COX/5-LOX inhibition emerged as an alternative and safe therapy for enhanced analgesic and anti-inflammatory effect with little or no gastric mucosal damage [8]. Recent studies [3] have also shown that dual inhibition is expected to curtail the

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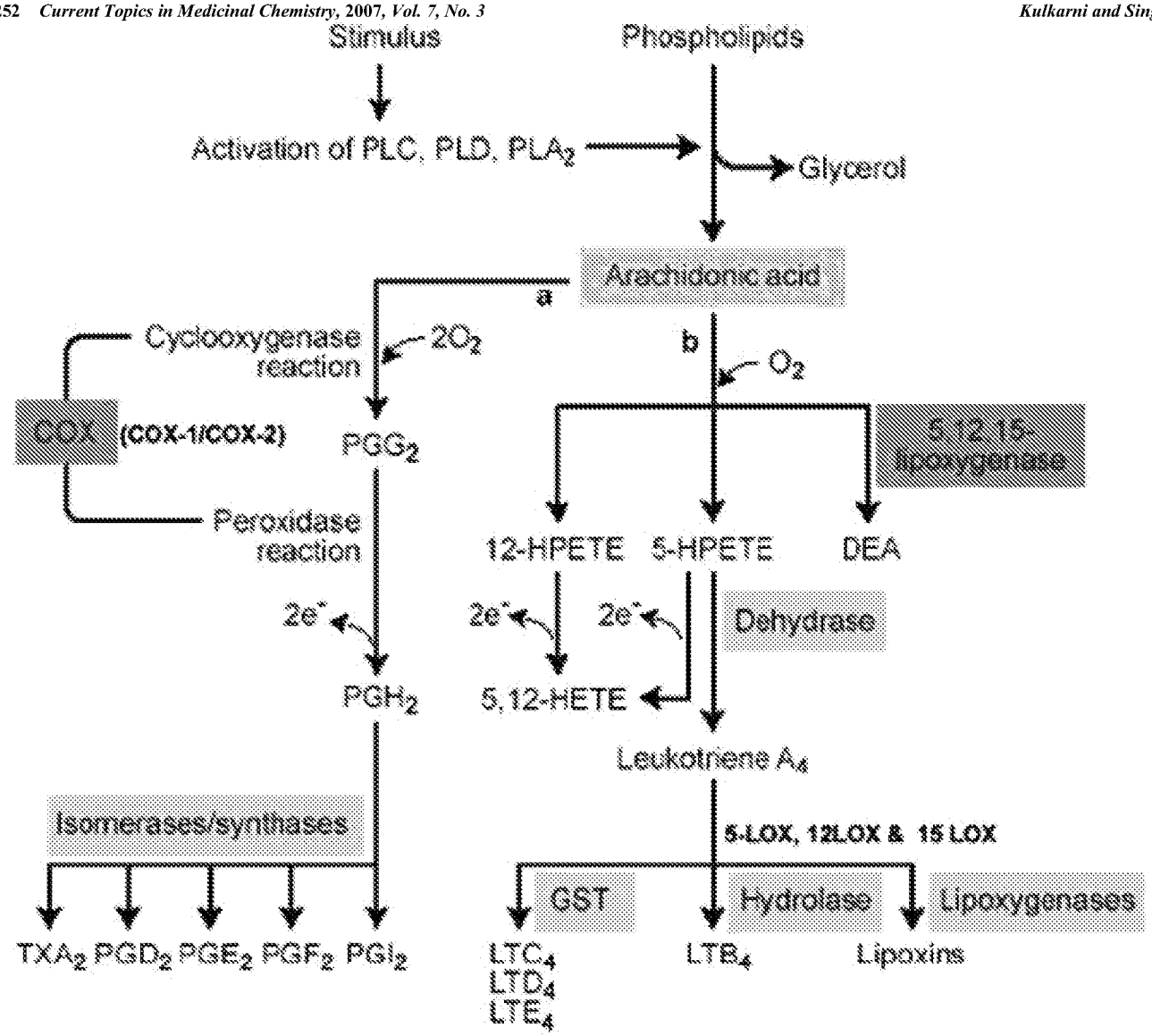


Fig. (1). The cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism. (a) The cyclooxygenase (COX) pathway results in the formation of prostaglandin G_2 (PGG_2) from arachidonic acid by a cyclooxygenase reaction. In a subsequent peroxidase reaction, PGG_2 undergoes reduction to prostaglandin H_2 (PGH_2). Both of these reactions are catalysed by COX (PGH synthase). PGH_2 serves as a substrate for cell-specific isomerases and synthases, producing prostanoids such as prostacyclin (PGI_2), thromboxane A_2 (TXA_2), $PGF_{2\alpha}$, PGE_2, and PGD_2 (b) The lipoxygenase pathways produce initially, hydroperoxyeicosatetraenoic acids (HPETEs) by lipoxygenases and subsequently convert these to (1) hydroxyeicosatetraenoic acids (HETEs) by peroxidases, (2) 5-LOX converts 5-HPETE into leukotriene-A_4 (LTA_4), (3) LTA_4 hydrolase converts LTA_4 into LTB_4, (4) LTC_4 synthase converts LTA_4 into LTC_4, and (5) LTC_4 is cleaved by glutathione S-transferases (GSTs) to LTD_4 and LTE_4.

adverse cardiovascular effects that are otherwise reported to be with COX-inhibition.

This present review focuses briefly on the patho-physiological role of LTs and on the experimental and clinical development of licofelone (ML3000), one of the most promising dual inhibitor of COX and 5-LOX.

PATHOPHYSIOLOGICAL ROLE OF LEUKO-TRIENES

AA can be converted to other metabolites, leukotrienes (LTs) by the enzymes called lipoxygenases, of which 5-LOX is the most important. 5-LOX is found in cells involved in the inflammatory process, such as monocytes, neutrophils, basophils, mastocytes, macrophages, B-lymphocytes and synoviocytes. It catalyzes the conversion of AA to leukotriene A_4 (LTA_4) via an unstable intermediate 5-hydroperoxyeicosatetraenoic acid (5-HPETE). Subsequently, LTA_4 is converted to leukotriene B_4 (LTB_4) or converted to cysteinyl leukotrienes (CysLTs) LTC_4, LTD_4 and LTE_4 [7] Fig. (**1**).

LTs modulate different regulatory processes of the microvasculature resulting into increase in margination of leukocytes, vascular permeability, and diapedesis of adherent leukocytes [9]. The major biological effect exerted by different LTs is summarized in Table **1**.

Table 1. Major Biological Effects of Leukotrienes

Leukotriene involved	Biological effect
LTB_4	Increased rolling and adhesion of leukocytes to endothelial cells
LTC_4, LTD_4, LTE_4	Increased vascular permeability (the contraction of adjacent endothelial cells and opening of tight junctions)
LTB_4	Stimulation of chemotaxis for neutrophils and to minor extent for other granulocytes, including eosinophils
LTD_4, LTE_4	Stimulation of chemotaxis for eosinophils in airway mucosa
LTB_4	Stimulation of secretion of superoxide anion and release of different granular constituents from leukocytes
LTB_4	Stimulation of IL-5, IL-6, and IL-8 release from T-lymphocytes
LTB_4	Increased expression of low-affinity receptors for IgE on B cell lines, and IgE synthesis induced by IL-4
LTC_4, LTD_4, LTE_4	Increased muscle tone and mucous secretions of the bronchial tree
LTC_4, LTD_4, LTE_4	Airway smooth muscle proliferation and remodeling

Among these, LTB_4 is most important mediators of inflammation. LTB_4 is a potent stimulator of leukocyte activation, and adhesion of these cells to vascular endothelium, elicits chemokinetic and chemotactic response. Exposure to LTB_4 causes recruitment of PMNs in tissues and exudates [10]. Apart from this, LTB_4 has also been shown to stimulate the production and release of proinflammatory cytokines from macrophage, lymphocytes and recently, from synovial membranes [11]. On the other hand, cysLTs contribute to inflammation by inducing vasoconstriction, and increase in post capillary venule permeability. This allows the leakage and further migration of fluid and proteins into inflammatory site [12].

Experimental trials have demonstrated that LTB_4 and CysLTs may participate in GI damage by inducing microvascular injury, gastric vessel vasoconstriction, promoting breakdown of the mucosal barrier and stimulating the secretion of gastric acid, as well as that of interleukin-1 and proinflammatory cytokines [13,14]. As confirmation to these findings, monoclonal antibodies directed towards CysLTs, receptor antagonists for LTB_4, and 5-LOX inhibitors prevented the effects on vascular tones and neutrophil-induced permeability [15]. Recently, it is reported that LTs may be responsible for the vascular permeability changes occurring during acute inflammation and may play a part in NSAIDs-induced GI damage due to shunt of AA metabolism towards the 5-LOX pathway when COX is inhibited [14]. In addition, increased expressions of 5-LOX protein and LTs in blood vessels have been observed following stimuli-evoked and aging-associated neurodegeneration [16].

The pathophysiological participation of LTs has been observed in various clinical conditions. Evidences suggested an elevated level of LTB_4 in blood and joint fluid from patients with rheumatoid arthritis. The biopsy samples from patients with lumbar herniated disc contain these biologically active substances [17,18]. An association between these materials and various types of lumbar disc herination is also reported. Kawakami *et al*., 2001 reported that relocation of autologus nucleus pulposus to lumbar nerve in rat produces time dependent and reversible hyperalgesia [19]. LTB_4 has also been found in inflamed colon mucosa in concentration known to induce deleterious effects (chemokinesis, cell aggregation, increasing vascular permeability) [20]. Recently, Zouboulis *et al*., 2003 reported potential involvement of LTB_4 in the development of acne where it regulates the cell proliferation, differentiation and apoptosis of sebaceous glands [21].

The biological properties of LTs, together with their formation in variety of diseases, suggest that 5-LOX inhibition may have therapeutic potential in allergic and inflammatory conditions. However, the results of animal studies and clinical trials were conflicting. Zileuton significantly reduced the allergen-induced nasal congestion when used with conventional drugs [22], but when tested in other conditions (rheumatoid arthritis and ulcerative colitis) [23,24], 5-LOX inhibitors have shown disappointing results. In animal studies, zileuton has shown protective effective against ulcerative colitis [25], whereas use of other LOX inhibitors (benoxaprofen and MK-591) did not offer meaningful advantage in relieving the inflammatory bowel disease [26]. Clinically, administration of zileuton for ulcerative colitis and rheumatoid arthritis was not found to be statistically different from placebo [24]. We reported effectiveness of 5-LOX inhibitors like zileuton in relieving herniated pain [27]. Further, zileuton, a 5-LOX inhibitor exhibited antinociceptive effect in paradigms of inflammatory pain suggesting the role of leukotrienes in other inflammatory conditions [28].

5-LOX inhibitors investigated to date for asthma have shown partial effectiveness allowing use of lower doses of conventional medications and have not shown clinical efficacy in other inflammatory disease. All these results seem to indicate that, the use of 5-LOX inhibitors have mixed outcomes in experimental animals and the clinical use of 5-LOX inhibitors might represent an insufficient single therapeutic model in inflammatory diseases other than asthma. Thus, it appears that discovery of the compounds that can block both the main metabolic pathways of AA metabolism is worthy of interest.

DUAL INHIBITORS OF 5-LIPOXYGENASE AND CYCLOOXYGENASES: BACKGROUND AND PHARMACOLOGICAL RATIONALE

An interesting paper published in 1996 by Nickerson-Nutter and Medvedeff observed that combination of leukotriene synthesis inhibitor and naproxen, a non-selective COX inhibitor, produced a significant reduction in the collagen-induced arthritis in mouse. No compound alone produced any inhibition of arthritis [29]. This observation was the beginning of a new era in the therapeutic approach i.e. inhibitors of COX and 5-LOX but not the inhibition of either enzyme in the management of inflammatory disorders.

Both conventional NSAIDs and the selective COX-2 inhibitors primarily exert their effect by reducing the production of PGs induced during an inflammatory event. In the recent years it has been clarified that, along with PGs, other key lipid mediators; LTs and lipoxins (LXs) complimentarily participate in development and persistence of inflammatory process. Since LTs are non-responsive to the effect of NSAIDs, it is suggested that the simultaneous dual inhibition of COX and 5-LOX pathways might have synergistic effects and achieve optimal wider spectrum of anti-inflammatory effect [30]. Recent studies have demonstrated that in the presence of COX (COX-1/-2 alone or combined) inhibition, LTs (LTB_4 and CysLTs) generated via shunt of AA towards 5-LOX pathway induces chemotaxis and local vasoconstriction that decreases the local blood flow to gastric mucosa [31]. Thus, the dual inhibitors of COX and 5-LOX may have two theoretical advantages of stronger anti-inflammatory and a protective effect on GI mucosa. In addition to this, 5-LOX/COX dual inhibition does not block the 12-or 15-LOX pathways, which together contribute by platelet/leukocyte cell-cell interaction to the production of lipoxins (LXs) that act as endogenous anti-inflammatory mediators of inflammation [30].

Apart from these two advantages the dual inhibition approach of inhibiting COX-1, COX-2 and 5-LOX might also provide additional cardioprotection. Adhesion of platelets and leukocytes on endothelial cells constitute an early mechanism of vascular inflammatory damage and consequent vessel occlusion. Pro-aggregatory and vasoconstrictor thromboxane A_2 (TXA_2) synthesised by COX-1 in platelets and cystLTs synthesized by 5-LOX in neutrophils, eosinophils and by cell-cell interaction contribute to coronary vasoconstriction. Once activated, leukocytes and platelet form platelet –leukocyte aggregates within the injured regions of vasculature to induce chemokine synthesis and respiratory burst of neutrophils. Under such circumstances, selective inhibition of either COX-1 or COX–2 or 5-LOX might not be effective in downregulating the vascular inflammation and thrombosis. It is therefore, suggested that inhibition of 5-LOX enzyme together with COX-1 and COX-2 would result into (i) inhibition of COX-1 to prevent platelet-derived TXA_2 formation; (ii) inhibition of COX-2 to downregulate leukocyte activation and widespread vascular inflammation; (iii) inhibition of 5-LOX to further, and specifically, reduce leukocyte inflammatory and thrombogenic potential, and to counteract the gastric damage associated with the inhibition of COX-1 [32]. The advancement in understanding the role and targeting these enzyme systems suggest a promising pharmacological approach in post-COX-2 era to reduce the adverse cardiovascular events (pro-thrombotic risk) of selective COX-2 inhibition without gastric side effects of non-specific inhibition of cyclooxygenase. Not only this, recent observations have highlighted a possibility that dual inhibitors of 5-LOX/COX might be also free from the acute renal toxicity, a side effect shared by available NSAIDs. If this is the case, development of dual inhibitors of 5-LOX and COX will offer an additional advantage over existing NSAID therapy, a proposition awaiting investigation.

Several chemically distinct compounds in 5-LOX/COX inhibitor category have been synthesized. Benoxaprofen was the first compound in this group, which was withdrawn from use in 1982 due to its hepatotopxicity and photosensitizing potential [33]. Other dual inhibitors such as BW 755C or SK&F 86002, have proved experimentally effective in preventing the production of both PGs and LTs and the conesquent inhibition of migration and activation of inflammatory cells (polymorphonuclear; PMN and macrophages) into inflamed sites. These compounds effectively prolonged the time of rejection of implanted polyester sponges in rats to day 22 compared with day 12 in naïve animals. Classical NSAID indomethacin did not change the rejection time [34].

Another orally active dual inhibitor ER-34122 also demonstrated interesting activity profile. ER-31422 demonstrated pronounced anti-inflammatory activity in a model of AA-induced ear inflammation in mouse. In addition to COX-inhibitory effect, it also demonstrated the LOX-inhibiting activity (inhibition of PMN leukocyte infiltration) [35]. The compound also showed anti-inflammatory activity in the early stages of spontaneous arthritis in MRL/MpJ-Ipr/Ipr mice and suppressed the progression of PMN infiltration, subsynovial soft tissue oedema, and multiplication of synovial lining cells in early stages of arthritis [36].

Tepoxalin (Zubrin™) by Schering plough, is a potent dual inhibitor of 5-LOX and COX-1/-2. It is now available as a rapid disintegrating tablet for use in dogs only. In experimental studies, tepoxalin has shown potent anti-inflammatory studies with an excellent gastric pattern over longer periods with higher doses [37]. In addition, pretreatment with tepoxalin prevented the indomethacin-induced early and late gastrointestinal damage. It also reduced the LTB_4 levels in stomach with no significant changes in PG levels [38]. Some studies in humans have demonstrated that oral administration of tepoxalin inhibits both COX and LOX and that single dose up to 800 mg or multiple doses up to 400 mg were well tolerated [39].

In spite of the promising results in experimental and few clinical trials, their further development for human use has been abandoned owing to liver toxicity. It is imperative to highlight that the hepatotoxicity is not due to their pharmacological mode of action, in fact is ascribed to the common molecular feature i.e. either due to broad inhibition of redox enzymes or presence of di-t-butyl moiety or hydroxamic acid [40].

The last generation of such compounds is devoid of redox potential and exhibited a dual and well-balanced inhibitory action on 5-LOX and COX by acting as a

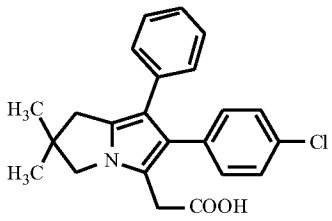
Table 2. Dual Inhibitors of COX and 5-LOX with their IC_{50} Values and *In Vivo*-Activity

	In vitro IC_{50} (μM)			
Compound	**COX-1**	**COX-2**	**5-LOX**	***In vivo* activity (ED_{50})**
Tepoxalin (Zubrin™)	0.80		0.15	Adjuvant induced arthritis in rats (3 mg/kg)
Tebufelone	1.50	0.02	20-22	Anti-inflammatory; antipyretic in humans (50mg vs. 600mg aspirin)
di-7-tert-butyl-2,3-dihydro-3, 3-dimethylbenzofuran (DHDMBF)	0.06	0.02	6.0	Anti-inflammatory
Darbufelone	Inactive	0.48	-	Well tolerated in human volunteers upto 100mg/day
BF389	-	0.84	3.65	Adjuvant arthritis in rat, paw oedema (1.2 mg/kg for oedema inhibition and 1.7 mg/kg for bone damage inhibition)
RJW63556	>10.0	1.81	1.02	Adjuvant arthritis in rat, paw oedema (3mg/kg)
PGV20229	7.00	0.22	8.00	Adjuvant arthritis in rat, paw oedema (6.6mg/kg)
C-1986	0.80		2.8	Carrageenan-induced foot paw oedema

substrate competitor. Table **2** provides some of potential dual inhibitors developed with their brief pharmacological profile. Darbufelone and licofelone are in phase-III clinical trials. The review will focus on pharmacological properties of licofelone, the extensively studied dual inhibitor of 5-LOX/COX in recent years.

LICOFELONE (ML3000)

Licofelone Fig. (**2**), ([2,2–dimethyl–6-(4-chloropheny-7-phenyl-2,3-dihydro-1H-pyrrazoline-5-yl] acetic acid) is the most promising of the dual inhibitors of 5-LOX/ COX inhibition. Originally discovered by Merckle GmbH and developed by EuroAllaince (a consortium of Alfa Wassermann SpA, Lacer SA and Merckle), it is presently under clinical evaluation (Phase III) for treatment of osteoarthritis (OA).



LICOFELONE

Originator	Merckle Gmbh
Licencees	Alfa Wassremann Spa, Lacer SA
Indications	Inflammation, osteoarthritis, pain
Actions	5-lipoxygenase/cyclooxygenase inhibitor, analgesic, antinflammatory
Synonym	ML 3000

Fig. (2). Structure of licofelone

In Vitro Effects on Eicosanoid Synthesis

The 5-LOX as well as COX-1/-2 inhibiting activity has been demonstrated in *in-vitro* studies. The 5-LOX and COX inhibitory activity of licofelone was firstly determined in bovine thromobocyte intact cell assay and intact bovine PMN leukocytes, respectively (IC_{50} COX=0.21 μM, IC_{50} LOX=0.18μM). Licofelone (0.3, 1, 10 and 30 μg/ml) showed a concentration-dependent inhibition of PGE_2 in human whole blood assay (IC_{50}=3.9μM vs. indomethacin 4.5μM). Further, licofelone was found to inhibit LTB_4 synthesis (IC_{50} =3.6μM) in basophilic leukemia cell assay using RBL-1 cell and LTC_4 synthesis in mixed PMN leukocyte/platelet stimulated with A-23187 (IC_{50}=3.8μM) [8]. Licofelone also inhibited in-vitro generation of reactive oxygen species, release of elastase by PMN leukocytes, homotypic PMN leukocyte aggregation stimulated by N-formyl-methionyl-leucyl-phenyl-alanine (fMLP), complement fraction 5a (C5a) and platelet activating factor (PAF), respectively [42]. These studies showed that licofelone had an inhibitory effect on COX-1/-2 and 5-LOX.

EXPERIMENTAL DATA IN ANIMALS

General Pharmacology

Licofelone was well tolerated, with no noticeable general behavioral effects when tested in animals. Licofelone up to 300 mg/kg (30, 100, and 300 mg/kg) did not affect any parameter in the Irwin test, locomotor activity, or hexobarbital-induced sleep. Intraduodenal administration of licofelone (100mg/kg) had no notable effects on the cardiovascular system or respiration in anesthetized rats or dogs or on neuromuscular function in anesthetized cats. No evidence of gastric damage or disturbances in perstalsis was observed with oral administration of licofelone. *In vivo*, licofelone dose-dependently inhibited acetylcholine, barium chloride or histamine-induced spasmogenic responses in guinea pig ileum. A small transient decrease in urine volume and electrolyte excretion was observed with highest dose of licofelone (300mg/kg) in rats [43].

Pharmacodynamic Activity

The pharmacodynamic profile of licofelone has been assessed in various animal models and compared with the effects of commonly used NSAIDs. The anti-inflammatory and anti-hyperalgesic effect of licofelone (30, and 100 mg/kg, po) was statistically significant ($p<0.05$) when compared to indomethacin (10 mg/kg, po) against carrageenan-, bradykinin-, and arachidonic acid-induced rat hind paw oedema. The ED_{50} value of 19.1 mg/kg (onset by 2h, duration: short), 13.0 mg/kg, and 16.8 mg/kg (onset by 1h, duration: long) was observed for licofelone against carrageenan-, arachidonic acid-and bradykinin-induced paw oedema, respectively. Similarly, licofelone showed ED_{50} value of 47.6 mg/kg (onset by 1h, duration: long), 92.2 mg/kg (onset by 1h, duration: medium), and 78.6 mg/kg (onset by 2h, duration: medium) against carrageenan-, arachidonic acid-and bradykinin-induced mechanical hyperalgesia, respectively. The percent inhibition and percent reversal against inflammation and mechanical hyperalgesia by licofelone was found to be greater than indomethacin (Table **3**). Also, licofelone (10-100 mg/kg, po) significantly ($p<0.05$) and dose-dependently prevented the Freund's adjuvant-induced increased vascularity in mice (vascularity index; 10 mg/kg: 0.059 ± 0.015; 20 mg/kg:0.048 ± 0.004, 30 mg/kg: 0.039 ± 0.012, 100mg/kg: 0.025 ± 0.015 vs. control: 0.0285 ± 0.003) [44]

In acetic acid (10ml/kg, ip, 1%v/v)-induced writhing, oral administration of licofelone (10, 30, 100 mg/kg, po) showed a significant decrease in incidence of writhings in mice (ED_{50} = 31.33 mg/kg). The time response curve with ED_{50} dose showed an onset of action after 15 minute post administration with peak effect at 30 min. The maximal possible effect (%MPE) at 30 min was statistically not different with that at 1 h (%MPE 30 min=44.91 ± 4.27 *vs.* 1h= 44.6 ± 3.49) (Singh *et al*., unpublished observation). A single dose of licofelone (10mg/kg, po) was found to be more effective than aspirin (50mg/kg, po) in mouse phenylquinone-induced writhing [45].

When tested in rat model of incisional pain (a model of post-operative pain), licofelone showed a longer duration of action and more effective than indomethacin and zileuton with ED_{50} values of 2.92 mg/kg, po and 36.77 mg/kg, po, in the mechanical hyperalgesia and cold allodynia testing, respectively Fig. (**3a** & **b**) [46].

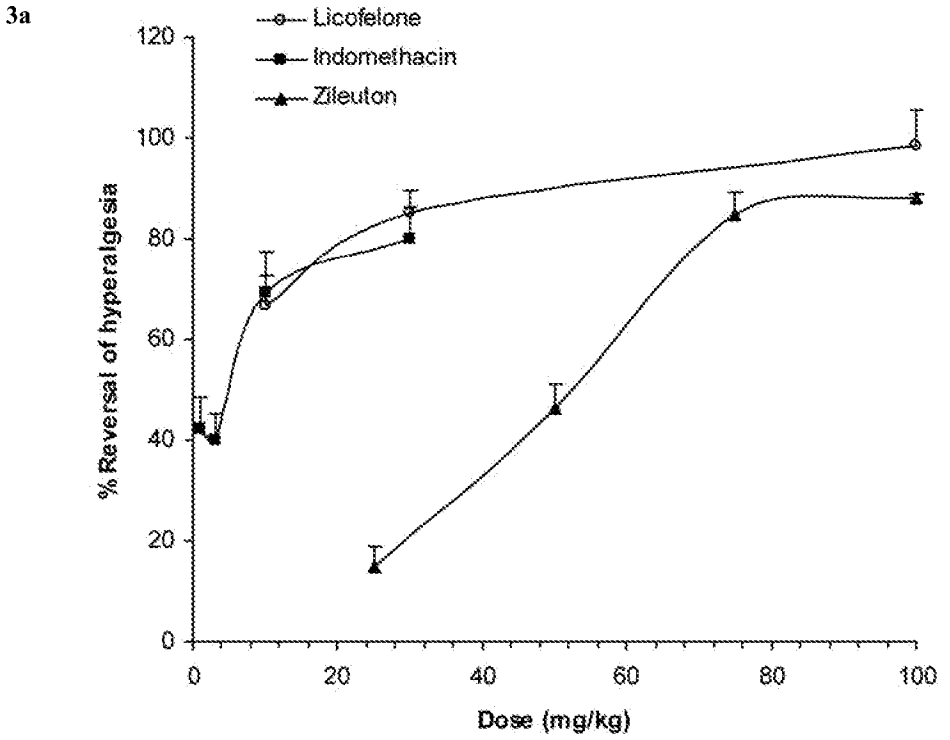
Tries and Laufer, 2001 observed an antipyretic effect of licofelone against brewer's yeast-induced hyperthermia in mice. The effect lasted over 3 hrs and was comparable to indomethacin (10mg/kg) [45]. Further, licofelone was highly effective and potent in a guinea pig model of broncho-constriction (ED_{50} = 0.2 mg/kg, iv), allergen-challenge in sheep, and also attenuated airway hyper-responsiveness to aerosolized carbachol [47].

The antithrombotic activity of licofelone (10, 30 and 100mg/kg, po) was comparable to aspirin (30, 100 mg/kg po) in rat laser-induced thrombosis. An *in vitro*-study demonstrated that licofelone (1-100μg/ml) had a marked inhibitory effect on platelet aggregation [48].

Table 3. Summary of *In-Vivo* Potency of Licofelone Against Inflammogen-Induced Paw Oedema (a) and Mechanical Hyperalgesia (b) in Rat [44]

(a) PAW OEDEMA							
Inflammogen	**Dose (mg/kg) of Licofelone**	**Route**	**MED (mg/kg)**	**Onset**	**Duration of action**	**Maximum percent reversal**	**ED_{50} (mg/kg) [CL 95%]**
Carrageenan	2, 10, 30, 100	po	10	2 h	Short	91.8 (4 h)	19.1 [11.2-27.0]
Arachidonic acid	2, 10, 30, 100	po	30	1 h	Long	90.7 (2 h)	13.0 [10.1-30.3]
Bradykinin	2, 10, 30, 100	po	30	I h	Long	93.9 (1 h)	16.8 [11.1-26.1]
(b) MECHANICAL HYPERALGESIA							
Carrageenan	10, 30, 100	po	30	1 h	Long	61.5 (4 h)	47.6 [39.5-55.8]
Arachidonic acid	10, 30, 100	po	10	1 h	Medium	55.0 (1 h)	92.2 [76.1-108-3]
Bradykinin	10, 30, 100	po	100	2 h	Medium	69.6 (4 h)	78.6 [53.9-103.3]

MED is defined as minimum dose that elicits a statistically significant reversal as compared to vehicle-treated controls. *po*-peroral.



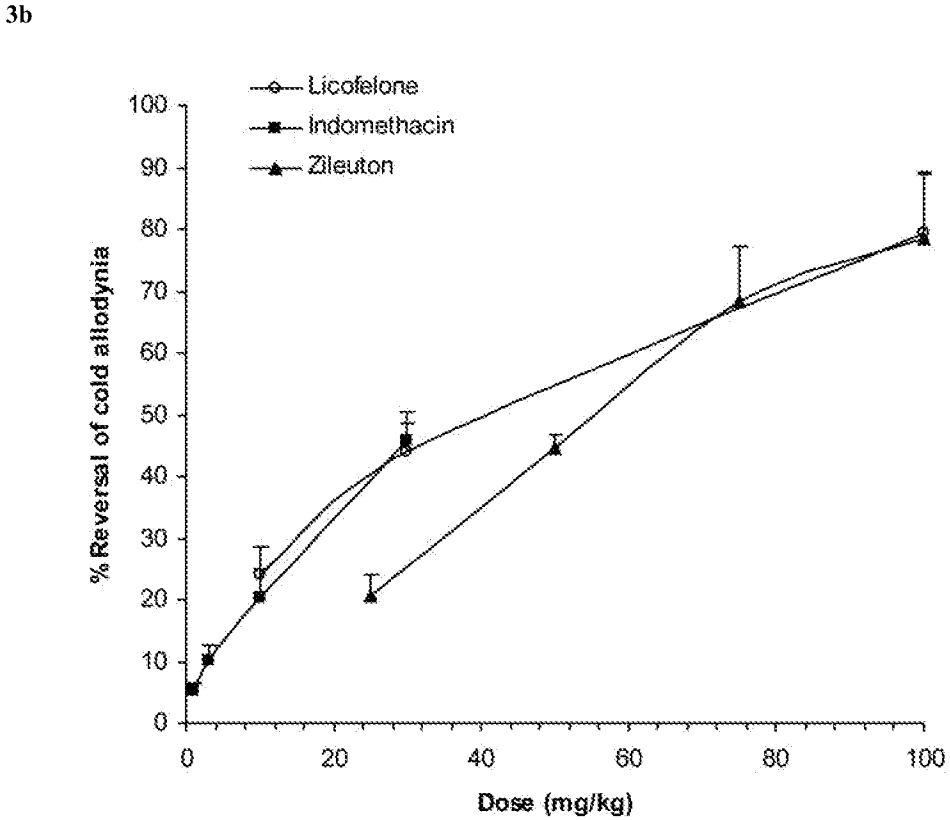


Fig. (3). Effect of licofelone (10-100 mg/kg, po), zileuton (25-100mg/kg, po), and indomethacin (1-30 mg/kg, po) on (a) reversal of incision-induced mechanical hyperalgesia, (b) reversal of incision-induced cold allodynia in the incised rat paw. Data (mean ± SEM) at the time of maximal reversal for each compound [46].

Jovanovic *et al*., 2001 reported that licofelone (20 or 80 mg/kg/bid for 28 days) significantly reduced the arthritis-associated deficiency of body growth, the oedema/erythema score, and splenomegaly in osteoarthritic (OA) dog. It also reduced the overall bone/cartilage erosions, histopathological score and also inhibited the appearance of fibroproliferative pannus in the ankle joint of arthritic dogs. Licofelone (2.5 and 5 mg/kg/day for 8 weeks) produced a marked reduction in the LTB_4 content of synovial fluid, IL-1β levels in synovial membrane, and cartilage collagen content. *In vitro*, licofelone inhibited the production of PGE_2, and LTB_4 by arthritic osteoblasts [49]. A recent study by Lajeunesse *et al*., 2004 reported prevention and/or delay in abnormal metabolism of subchondral osteoblasts in experimental dog osteoarthritis (OA) with licofelone. Licofelone also reduced PGE_2 levels in osteoblast cell cultures and decreased uro-kinase plasminogen activator and insulin like growth factor-1 [50].

The major pharmacodynamic studies of licofelone are summarized in Table **4**.

Pharmacokinetics

The pharmacokinetic profile of licofelone was studied in male rats using LC/MS/MS {mass spectrometer API 3000 (PE SCIEX, Concord, ON Canada), fitted with a turbo ion spray as ion source, Lichnocart, RP-18, 40X30 mm, 3mm column under isocratic conditions, mobile phase (90: 10 methanol/0.2% formic acid, 0.2ml/min)}. After a single dose of licofelone (100mg/kg, po) plasma levels of licofelone peaked at 2-4 h (t_{max}), with maximum plasma concentration 17.63 ± 3.2μg/ml (C_{max}), and $t_{1/2}$ = 10-11h (Singh *et al*., unpublished observation). Deigner *et al*., 1995 studied the plasma levels and distribution ^{14}C-labeled licofelone (13.7-26.6 mg/kg) in female rats. A plasma levels of licofelone peaked at 3-4 h post administration with a plasma $t_{1/2}$ of ~11h. The highest concentration of licofelone was found in liver, lung, kidney, heart and intestine [51]. Almost no penetration of the blood-brain barrier was found; however, after 48 h there was minor accumulation in fats. Upon chronic (14 day) administration of licofelone (10mg/kg, po) to male rats a steady state concentration of licofelone was observed in systemic circulation after day 5 onwards till day 14. The concentration of licofelone was about 96 times *in vitro* IC50 of licofelone for 5-LOX and 82 times that for COX-1/-2 (Singh *et al*., unpublished observation).

Toxicity (LD_{50}, and Effect on GI and Kidney)

No toxic symptoms were observed with a single dose of licofelone (300 mg/kg po or 100mg/kg ip) in ICR mice. The LD_{50} of licofelone on oral administration was calculated in both male and female Swiss mice and Wistar rats. Both mice and rats were dosed with licofelone (aqueous suspension in 2% Tween 80) 500, 1000, 1500, and 2000 mg/kg, po and observed for any toxic symptoms or mortality for 4h post dosing and thereafter once daily for 14 days. Both male and female mice/rats administered with high doses of 1500 and 2000 mg/kg licofelone showed toxic symptoms of stupor, and were moribund from day 5 onwards. Licofelone showed 40% and 60% mortality in both male and female mice at a dose of 1500 and 2000mg/kg po, respectively. In Wistar rats, licofelone showed a mortality of 60% and 80% in both male and female rats at a dose of 1500 and 2000 mg/kg, po, respectively. The LD_{50} calculated from graphical method was found to be 1970.17 mg/kg, po for both male and female Swiss mice, where as LD_{50} of licofelone was found to be 1473.16 mg/kg, po in case of both male and female Wistar rats (Singh *et al*., unpublished observation). Licofelone did not demonstrate genotoxic potential in bacteria and mammalian cells *in vivo* [52].

The gastrointestinal (GI) tolerability of licofelone has been examined in rats and rabbits. Licofelone (10 to 100mg/kg, po) did not produce any acute damage; however, 5 day treatment with the same dose produced slight non-significant GI damage. Some of the rats dosed with 30 and 100 mg/kg of licofelone for 11 days showed presence of duodenal ulcers [53,54]. In rabbits, repeated administration of licofelone (10, 30 mg/kg) did not produce any gastric damage. Conversely, repeated administration of diclofenac (20mg/kg, po) to rabbits resulted in formation of antral ulcers in about 80% of the rabbits. However, an antral ulcer occurred in one of the rabbit that received licofelone (100mg/kg, po). The weak gastrotoxic potential of licofelone was linked to its mechanism of action, whereby in the presence of inhibition of PG synthesis in stomach, it prevented the increase in LTB_4 synthesis, a key factor in NSIAD-induced GI damage [55]. Acute Pretreatment with licofelone (30mg/kg, po) reversed indomethacin (100mg/kg, po)-induced gastric ulceration, neutrophil adhesion in mesentry venules, neutrophil count in blood, and lipid vascularity in rats. Further, the chronic pretreatment with licofelone (10mg/kg, po) also prevented indomethacin (2mg/kg, po)-induced gastric morphological changes, and cellular infiltration in mesentry venules of rats. Licofelone was also found to be effective in reducing indomethacin–induced vascular permeability in mouse, whereby it effectively prevented the leakage of dye in stomach [vascularity index indomethacin (10mg/kg, po= 0.071± 0.003; licofelone (10mg/kg, po) =0.030 ± 0.005)] [56]. Licofelone per se did not induce an increase in LTB_4 content of rat gastric mucosa. Smoloka *et al*., 2004, studied *in vitro* the mechanisms underlying gastric sparing activity of licofelone. Licofelone reversibly inhibited SCH28080-sensitive H,K-ATPase activity in pig gastric microsomes (IC50 = 16.4 μM). It also dose-dependently inhibited histamine-stimulated acid accumulation (IC50 = 40μM), and forsklin-stimulated acid accumulation (IC_{50}=45μM). Lastly, in human gastric adenocarcinoma cells, licofelone dose-dependently inhibited both baseline and interleukin-1β-stimulated interleukin 8 secretion with IC_{50} of 0.46 μM and 1.1 μM, respectively [57].

Further experiments have shown that licofelone administered to aspirin-treated rats did not exacerbate acute gastric injury. In contrast, conventional NSAID, indomethacin or a selective COX-2 inhibitor, celecoxib exacerbate acute gastric injury [58]. This was attributed to 5-LOX inhibiting property of licofelone.

Furthermore, the effect of single dose of licofelone (30, 100, and 300mg/kg) produced a slight decrease in the urine output of rats. The effect was significant during the first hour following highest dose. A dose-related inhibition of

Table 4. Summary of Pharmacodynamic Activity of Licofelone

	In vitro IC_{50} (μM)			
Compound	**COX-1**	**COX-2**	**5-LOX**	
Licofelone (ML3000)	0.16-0.2		0.1-0.23	
PHARMACODYNAMIC ACTIVITY				
Study type	**Effect studied**	**Experimental model**	**Result**	**Reference**
In-vivo	Anti-inflammatory	Carrageenan-induced rat paw oedema	ED_{50} = 19.1 mg/kg, po (onset 2h, short duration)	[44]
		Bradykinin-induced rat paw oedema	ED_{50} = 13.0 mg/kg, po (onset 1h, long duration)	[44]
		Archidonic acid-induced rat paw oedema	ED_{50} = 16.8 mg/kg, po (onset 1h, long duration)	[44]
		Freund's adjuvant-induced acute inflammation	ED_{50} = 18.2 mg/kg po	Singh *et al.*, unpublished observation
		Freund's adjuvant-induced established inflammation	ED_{50} = 17.5 mg/kg, po	Singh *et al.*, unpublished observation
		Rat adjuvant- arthritis	Licofelone (10mg/kg) significantly decreased the secondary lesion	[43]
In-vivo	Analgesic	Acetic acid-induced writhing	ED_{50} 31.33 mg/kg, po	Singh *et al.*, unpublished observation
		phenylquinone-induced writhing in mice.	Licofelone 10 mg/kg, po was more effective than aspirin 50 mg/kg, po	[45]
In-vivo	Anti-hyperalgesic effect	Carrageenan-induced rat hind paw mechanical hyperalgesia	ED_{50} = 47.6 mg/kg, po (onset 1h, long duration)	[44]
		Bradykinin-induced rat hind paw mechanical hyperalgesia	ED_{50} = 78.6 mg/kg, po (onset 2h, medium duration)	[44]
		Arachidonic-induced rat hind paw mechanical hyperalgesia	ED_{50} = 92.2 mg/kg, po (onset 1h, medium duration)	[44]
		Freund's adjuvant-induced acute hyperalgesia	4.6 mg/kg, po	Singh *et al.*, unpublished observation
		Freund's adjuvant-induced established inflammation	12.0 mg/kg, po	Singh *et al.*, unpublished observation
		Incisional pain in rats	2.92 mg/kg, po (onset 3h, long duration)	[46]
In-vivo	Anti-allodynic effect	Incisional pain in rats	36.77 mg/kg, po (onset 1h, long duration)	[46]
In-vivo	Antipyretic	Brewer's yeast-induced hyperthermia in rats	Licofelone (10mg/kg) showed a significant analgesic effect over 3h	[45]
In-vivo	Anti-thrombic	Laser-induced thrombus in rats	Licofelone (10, 30, 100 mg/kg, po) demonstrated significant anti-thrombotic effect, comparable to aspirin	[48]
In-vivo	Disease-modifying	Osteoarthritic dogs	Licofelone (2.5, 5 mg/kg, po for 8 weeks) significantly decreased the size, grade and severity of cartilage lesions in condyles and plateaus	[49]
			Delayed abnormal metabolism of subchondral osteoblasts. Also reduced PGE_2 levels in osteoblast cell cultures and decreased urokinase plasminogen activator and insulin like growth factor-1	[50]

(Table 4) Contd....

PHARMACODYNAMIC ACTIVITY				
Study type	**Effect studied**	**Experimental model**	**Result**	**Reference**
In-vivo	Gastroprotective	NSAIDs-induced gastroinflammation	Pretreatment with licofelone prevented the NSAID-induced neutrophil accumulation in rat mesentry, inhibited increase in gastric LTB_4, reduced lipid peroxidation and vascular permeability	[56]
			Licofelone coadministered to aspirin-treated rats did not exacerbate acute gastric injury and also did not alter the gastric adaptation to aspirin	[58]

electrolytes was observed following 100 mg/kg (k^+ and Cl^-) and 300 mg/kg (Na^+, K^+ and Cl^-) [43].

In summary, the pharmacological data (general, pharmacodynamics, and toxicity) in various animal experiments indicated that licofelone has antipyretic, analgesic, anti-inflammatory, anti-platelet activity of NSAIDs, and probably their renal side effects, yet gastrofriendly and possibly cardioprotective. However, clinical studies are needed to investigate the actual relevance of these studies and to establish benefit to risk ratio in the end users, i.e. humans.

CLINICAL STUDIES

Clinical Pharmacokinetics

Till date, the clinical pharmacokinetics of licofelone has been poorly investigated. One study compared the pharmacokinetic parameter of licofelone (200mg bid for 5 days and a single final dose of 200 mg on day 6) in male and female, young (mean age 30.9 yr) and elderly (mean age 72.1 yr) healthy volunteers. After the first dose, the mean C_{max} for both young and elderly was similar (young 1.66 ± 1.1 μg/ml; elderly 1.63 ± 0.9μg/ml), however the mean $AUC_{0\text{-}12}$ was 23% lower in young population (5.64 ± 2.0μg/ml; elderly 4.58 ± 1.92 μg/ml). Although, steady state was achieved in both the age groups with approximately similar C_{max}, the AUC was 20% higher in elderly individuals, $t_{1/2}$ (β) greater in young population (11.1 ± 7.1 *vs.* 8.7 ± 4.7 h in elderly), and $t_{1/2}(\alpha)$ 15 % high in elderly population [59]. Further, in another study, no interaction between warfarin and licofelone was observed. Different metabolic pathways may explain the absence of pharmacokinetic interaction between licofelone and warfarin. Warfarin is predominantly metabolized by cytochrome P450-caytalyzed hydroxylation, whereas licofelone is mainly by glucouronidation and CYP 34A, 2D6, and 2C19-catalyzed hydroxylation [60].

Clinical Trials

The reports of extensive detailed clinical trials data is not in the public domain as yet and the outcome of clinical trials are available either as press releases of abstracts of the two Annual European Congress of Rheumatology (EULAR 2002 and EULAR 2003).

PHASE I

The phase I clinical study established the safety and tolerability of licofelone 200 or 400mg, bid, daily for 4 weeks, in 121 healthy volunteers in comparison to naproxen 500 mg or placebo. At the end of treatment period, the modified Lanza scores for gastric mucosal damage in licofelone group were similar to placebo and statistically less than that of naproxen (licofelone 200mg 0.13± 0.57; licofelone 400mg 0.14 ± 0.45; placebo 0.20 ± 0.66 and naproxen 500 mg 1.47± 1.31). However, the duodenal scores were not different among the groups. The gastric mucosa was normal in 93% of the subjects who took licofelone 200 mg, 89% in those treated with licofelone 400mg, 90% in case of placebo and 37% for naproxen group. No ulcers were present in either of licofelone or placebo group, but 20% of the naproxen subjects presented ulcers [61,62]. Accordingly, the gastrotolerability of licofelone was found to be superior than that of a nonselective NSAID, naproxen.

PHASE II

Two double-blind, randomized, placebo controlled, phase-II studies tested the efficacy of licofelone in OA. In the first study, 107 patients received licofelone (100, 200 or 400 mg/kg) or placebo for 4 weeks. Doses of 200 or 400mg twice a day were effective at relieving symptoms (pain and stiffness), as determined by WOMAC index. In a second study, 404 patients treated with licofelone 100, 200, or 400mg, bid, showed a mean percent decrease of 37, 40, and 42, respectively in WOMAC pain subscore. The mean percent decrease in subscore and improvement in secondary endpoints (pain, stiffness, and disability) was significantly greater in licofelone groups than placebo. The adverse events of diarrhea and abdominal pain were experienced with licofelone at 400mg bid [62, 63].

PHASE III

In Phase III clinical trials, OA patients (n=148) were treated with either licofelone (200mg), or naproxen (400mg), twice daily. WOMAC index was used to evaluate the efficacy in OA patients. Licofelone was found to be as effective as naproxen with 69.4% and 68.4% responders, respectively. However, the incidences of gastrointestinal adverse effects and gastrointestinal ulcers were higher in the naproxen group [64].

Long-Term Efficacy and Safety Studies

Long-term (52 week), double blind, efficacy study in 710 OA patients demonstrated that licofelone (100 or 200 mg bid) had an improved efficacy throughout the 52 weeks. In general and GI tolerability of licofelone was better than

naproxen (500mg, bid) (incidence of ulcers licofelone 100 mg bid 0.14%; licofelone 200mg bid 0.39% and naproxen 500mg bid 2.5%). Further, both the doses of licofelone were associated with lower incidence of hypertension aggravation (0.4%) as compared to naproxen (3.1%) [64].

In another randomized, double blind, 12 month clinical trial assessed the safety and tolerability of licofelone 100mg (n=235), licofelone 200mg (n=240), and naproxen 500mg (n=229), twice daily, in patients with symptomatic OA of knees. Licofelone 400 mg/day was as effective as naproxen 1g/day throughout the study in the primary efficacy end point (WOMAC pain score). A dose-response effect was seen for licofelone. In naproxen group 69.7% patients complained of adverse events in comparison to 59.2% and 56.2 % of those receiving licofelone 200 and 400 mg/day, respectively. The incidence of GI adverse effects, peripheral oedema and hypertension aggravation was less with licofelone as compared to naproxen treatment [65].

Comparing Licofelone with Celecoxib

Pavelka *et al.*, 2003 reported a better efficacy and tolerability with licofelone 200mg bid in patient with OA of knees. Licofelone treatment (n= 302) was as effective as celecoxib 200mg (n=306) once daily. However, low adverse event rate was reported with licofelone (31.9%) than the celecoxib group (36.4%). No comparison of relative GI toxicity was conducted [66]. Finally, the results of another unpublished study in phase III were less favorable, possibly due to unexpectedly high placebo response [67].

GI Safety of Licofelone with Aspirin Co-Medication

Preliminary experimental data has suggested that licofelone retains the favorable GI profile when co-administered with low dose of aspirin. The GI tolerability of licofelone when co-administered with low dose of aspirin was studied in two multicentric, randomized, double blind, endoscopy trials. In one study, licofelone 400 mg (twice the therapeutic dose) or naproxen 500mg bid was administered with enteric-coated low dose aspirin 81 mg once daily (licofelone n=35, naproxen n=41) whereas other study had no concomitant use of aspirin (licofelone n=95, naproxen n=106). The superior GI tolerability of licofelone compared to naproxen was confirmed in both the studies. In the first study the GI ulcer index for licofelone or naproxen was 2.9 and 26.8%, respectively, and 2.1 and 20.8%, respectively in second study. The results indicated that licofelone retains excellent GI tolerability profile even when co-administered with low-dose of aspirin.

CONCLUSION

The experimental data for licofelone reviewed here, supports the development of dual inhibitors of 5-LOX/COX as an alternate therapy to currently available NSAIDs. The results of clinical studies indicate that licofelone is atleast as effective as naproxen for the treatment of OA, both in short- and long-term studies. However, licofelone showed a remarkable GI safety over naproxen. In addition, concomitant administration of licofelone with low dose of aspirin neither exacerbates the ulcerogenic profile of aspirin nor did it alter the adaptation of the gastric mucosa to aspirin. Furthermore, licofelone was also found to be as effective as celecoxib, with a similar GI tolerability. Nevertheless, the low rate of peripheral oedema observed with licofelone use indicated that licofelone could have an additional advantage over selective COX-2 inhibitors. Further investigations of the efficacy and GI tolerability of licofelone are ongoing. It has still not been studied in patients with inflammatory rheumatic diseases such as rheumatoid arthritis, and spondylarthropathies. Also, the GI tolerability of licofelone needs confirmation in patients predisposed to NSAID-induced ulcer, with history of peptic ulcers or those on corticosteroids. In addition, there is a need for large-scale trials to investigate the effect of licofelone on the upper GI events (symptomatic ulcers, bleeding, perforations) in comparison to selective COX-2 inhibitors. Investigations are also awaited for the use of licofelone in patients with low dose aspirin for cardiovascular prophylaxis and in patients with asthma and/or aspirin intolerance.

In conclusion, the experimental data strongly suggest that the licofelone by its inhibitory effect on 5-LOX, COX-1, and COX-2 causes a decrease in production of both LTs and PGs. Therefore, it has the potential to offer clinically relevant advantages of GI tolerability and peripheral oedema and efficacy (analgesic and anti-inflammatory activity) better than NSAIDs or selective COX-2 inhibitors. However, investigations in large number of patients are still needed to confirm the anti-inflammatory and analgesic effect in humans.

ABBREVIATIONS

5-LOX	=	5-Lipoxygenase
COX	=	Cyclooxygenase
CysLT	=	Cysteinyl leukotrienes
5-HPETE	=	5-Hydroperoxyeicosatetraenoic acid
NSAIDs	=	Non-steroidal anti-inflammatory drugs
PGs	=	Prostaglandins
LTs	=	Leukotrienes
PMN	=	Polymorphonuclear

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Global health care challenge: Indian experiences and new prescriptions*

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Globally, there has been an unparalleled growth in the plant-derived medicinally useful formulations, drugs and health-care products, its market covering more than 60% products derived from plant origin. India exhibits remarkable outlook in modern medicines that are based on natural products besides traditional system of Indian medicines. Almost, 70% modern medicines in India are derived from natural products. Medicinal plants play a central role not only as traditional medicines but also as trade commodities, meeting the demand of distant markets. Ironically, India has a very small share (1.6%) of this ever-growing global market. To compete with the growing market, there is urgency to expeditiously utilize and scientifically validate more medicinally useful plants while conserving these species, which seems a difficult task ahead. This paper begins with an overview of the value of Medicinal and Aromatic Plants and discusses its usefulness in the traditional medicines. Then it briefly assesses the potential of medicinally useful plants and prospects of modern medicines and health care products derived from plant origin and based on the knowledge of alternative system of medicine in India. It thereafter concisely touches upon India's varied biodiversity, comparative Research and Development strength, strong pharmaceutical manufacturing base and traditional wisdom in medicines to improve its market potential. In the conclusion, there are major recommendations to help India evolve as a major drugs and herbal based health care products leader in the world market.

MEDICINAL AND AROMATIC PLANTS: AN OVERVIEW

India has 2.4% of world's area with 8% of global bio-diversity. It is one of the 12 mega-diversity hot-spot regions of the world, other countries being Brazil, Colombia, China, South Africa, Mexico, Venezuela, Indonesia, Ecuador, Peru, USA and Bolivia. Across the country, the forests of India are estimated to harbour 90% of India's medicinal plants diversity in the wide range of forest types that occur. Only about 10% of the known medicinal plants of India are restricted to non-forest habitats. The estimated numbers of plant species and those used for medicinal purpose vary. According to Schippmann et al. (2002), one fifth of all the plants found in India are used for medicinal purpose. The world average stands at 12.5% while India has 20% plant species of medicinal value and which are in use Table 1. But according to Hamilton (2003), India has about 44% of flora, which is used medicinally (Table 2). Although it is difficult to estimate the number of medicinal and aromatic plants present worldwide, the fact remains true that India with rich biodiversity ranks first in per cent flora, which contain active medicinal ingredient.

MAPs AND TRADITIONAL MEDICINE

The existence of traditional medicine depends on plant species diversity and the related knowledge of their use as herbal medicine. In addition both plant species and traditional knowledge are important to the herbal medicine trade and the pharmaceutical industry whereby plants provide raw materials and the traditional knowledge prerequisite information (Tabuti et al. 2003).

India has one of the richest plant medical traditions in the world. It is a tradition that is of remarkable contemporary relevance for ensuring health security to the teeming millions. There are estimated to be around 25,000 effective plant-based formulations, used in folk medicine and known to rural communities in India. There are over 1.5 million practitioners of traditional medicinal system using medicinal plants in preventive, promotional and curative applications. It is estimated that there are over 7800 medicinal drug-manufacturing units in India, which consume about 2000 tonnes of herbs annually (Ramakrishnappa, 2002).

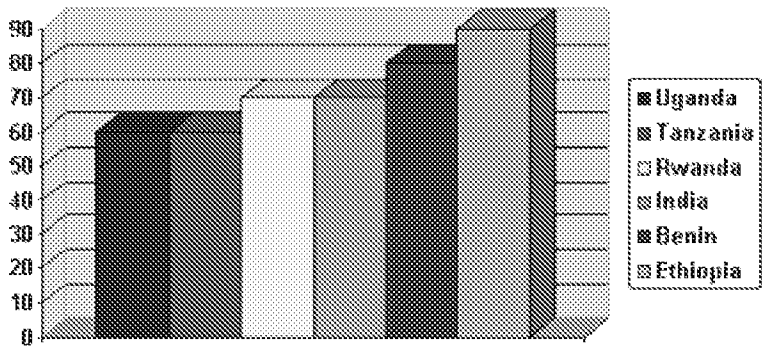

Figure 1. Percent use of traditional medicine for primary health care in few developing countries.
Source: WHO, 2002

The market for ayurvedic medicines is estimated to be expanding at 20% annually. Sales of medicinal plants have grown by nearly 25% in India in past ten years (1987-96), the highest rate of growth in the world (Masood, 1997). But the per capita expenditure in India on medicines per annum is amongst the lowest in the world. In other developing countries too, plants are the main source of medicine (Figure 1). Two of the largest users of medicinal plants are China and India. Traditional Chinese Medicine (TCM) uses over 5000 plant species; India uses about 7000. According to Export Import Bank, the international market for medicinal plant related trade is to the tune of US$ 60 billion having a growth rate of 7% per annum. China's share in world herbal market is US$ 6 billion while India's share is only US$1 billion which according to Rawat is 'expected to rise to Rs.3000 crores by 2005' (Rawat, 2002) while TCM is projected to rise to US$ 400 billion by 2010 (Wang and Ren, 2002).

Traditional and folklore medicine handed on from generation to generation is rich in household remedies and community practice. According to an estimate of World Health Organization (WHO), nearly 80% of the populations of developing countries rely on traditional medicine, mostly plant drugs for their primary health care needs.

Traditional medicine has served as a source of alternative medicine, new pharmaceuticals, and healthcare products. Medicinal plants are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds (Mukherjee, 2003).

A significant number of modern pharmaceutical drugs are thus based on or derived from medicinal plants. The derivatives of medicinal plants are non-narcotic with little or no side effects.

POTENTIAL AND PROSPECTS OF PLANT BASED MODERN MEDICINES AND HEALTH CARE PRODUCTS: CAN INDIA TARGET THE INTERNATIONAL MARKET?

It is estimated that nearly three fourths of the plant-derived prescription drugs used worldwide were discovered following leads from local medicine. About 25% of modern medicines are descended from plants first used traditionally according to WHO. Many others are synthetic analogues built on prototype compounds isolated from plants. Almost, 70% modern medicines in India are derived from natural products (Choudhary, 2002).

The basic uses of plants in medicine will continue in the future, as a source of therapeutic agents, and as raw material base for the extraction of semi-synthetic chemical compounds such as cosmetics, perfumes and food industries. Popularity of healthcare plant-derived products has been traced to their increasing acceptance and use in the cosmetic industry as well as to increasing public costs in the daily maintenance of personal health and well being. In the dual role as a source of healthcare and income, medicinal plants make an important contribution to the larger development process. Though the efficacy of herbals requires development of quality consciousness in respect of the evaluation related evidences, supplying the demand for botanicals and herbals is a booming business (Mukherjee, 2002).

Recently even developed countries, are using medicinal systems that involve the use of herbal drugs and remedies. Undoubtedly the demand for plant-derived products has increased worldwide. The demand is estimated to grow in the years to come fuelled by the growth of sales of herbal supplements and remedies according to several surveys. This means that scientists, doctors and pharmaceutical companies will be looking at countries like China, India,

Table 1. Numbers and Plants used medicinally worldwide (Schippmann et al. 2002).

Country	Plants species	Medicinal plant species	Percentage
China	26,092	4,941	18.9
India	15,000	3,000	20.0
Indonesia	22,500	1000	4.4
Malaysia	15,500	1,200	7.7
Nepal	6,973	700	10.0
Pakistan	4,950	300	6.1
Philippines	8,931	850	9.5
Sri Lanka	3,314	550	16.6
Thailand	11,625	1,800	15.5
USA	21,641	2,564	11.8
Vietnam	10,500	1,800	17.1
Average	13,366	1,700	12.5
World	422,000	52,885	

etc. for their requirements, as they have the most number of medicinal plant species and are the top exporters of medicinal plants.

WORLD MARKET: EXPORT OPPORTUNITIES FOR DEVELOPING COUNTRIES

The value of medicinal plants as a source of foreign exchange for developing countries depends on the use of plants as raw materials in the pharmaceutical industry. It provides numerous opportunities for developing nations to advance rural well being. The global trade in medicinal plants is of the order of US$ 800 million per year. Export statistics available between 1992 and 1995 indicate that India exported about 32,600 tonnes of crude drugs valued at $US 46 million (Dhar et al. 2002). China with exports of over 120,000 tons per annum (US$ 264.5 million) and India with over 32,000 tons per annum dominate the international market. The annual export of medicinal plants from India is valued at Rs. 1200 million (Ramakrishnappa, 2002). All the major herbal-based pharmaceutical companies are showing a constant growth of about 15 per cent or more, next only to Information Technology industry (Kumar, 2000). The turnover of herbal medicines in India as over-the-counter products, ethical and classical formulations and home remedies of Ayurveda, Unani, and Siddha systems of medicine is about US$1 billion with a meagre export of about US$ 80 million (Kamboj, 2000).

The worldwide market of herbal medicines is of the order of US$60 billion (WHO, 2002) to US$80 billion (Mathur, 2003). In the West the demand for herbal drugs has reached a new high in recent years. About 1400 herbal preparations are used widely according to a survey in Member States of European Union (Hoareau and DaSilva, 1999). In 1999 the global market for herbal supplements exceeded US$ 15 billion, with a US$ 7 billion market in Europe, US$ 2.4 billion in Japan, US$ 2.7 in the rest of Asia and US$ 3 billion in North America (Table 3). The use of herbal medicine is widespread, with as many as three in ten Americans using botanical remedies in a given year (Raskin et al. 2002).

In the USA, sales of botanical products (fortified foods, dietary supplements) increased by more than 40% between 1992 and 1996 to reach a global value of more than US$14 billion in 1996. Similar trends were observed in Japan and the Western European countries too (Schilter et al. 2003).

It is estimated that Europe imports about 400,000 tons of medicinal plants per annum, with an average market value of US$ 1 billion from Africa and Asia. Germany is by far the largest market and within Europe, largest consumer of medicinal plants, spending £ 1.4 billion (US$ 2.2 billion) annually. France is second (£ 116 million) and the United Kingdom third (£ 88 million) (Masood, 1997). Among the importers of botanical drugs, Hong Kong is at the top followed by Japan, Germany and USA. These assessments of international trade in medicinal plants include plants and their parts like roots, tubers, wood extract, bark, leaves, flowers, fruit and seeds. Germany and the USA are among the top four countries in import as well as export, expressing their major role as a turntable for medicinal plant raw materials worldwide.

Several important modern drugs are extracted directly from plants. It has been estimated that only 6% of all described species have been analysed chemically and only a small fraction analysed pharmacologically (Choudhary, 2002). In the USA, the process of synthetic drug discovery and development takes an average of 12 years, and any new drug requires the investment of an average of US$ 230 million. It is seen that plant based drugs take a comparatively much less time and expenses than synthetic drugs. Hence plant based medicines would be cheaper, unless the market price are inflated by other considerations (Ramakrishnappa, 2002).

Table 2. Numbers and percentage of medicinal plant species recorded for different countries and regions (Hamilton, 2003).

Country or Region	Total no. of native species of flora	No. of species of medicinal plants	% of flora which is medicinal
China	27,100	11,146	41
India	17,000	7,555	44
Mexico	30,000	2,237	7
North America	20,000	2,572	13
World	297,000-510,000	52,885	10-18

Some drugs are synthesized copies of chemicals found naturally in plants i.e. aspirin which is a safer synthetic analogue of salicylic acid, an active ingredient of willow bark. The market share of herbal products made in developing countries remains comparatively low due to lack of research and development and the huge investments in making standardized products. Extraction of active principles and manufacture of drug formulations is sophisticated technology and capital intensive. A systematic and a concerted approach to this activity have not been maintained for want of sophisticated equipment and high-cost chemicals. Even in India, there has been a lack of Research and Development on product and process development although recent research has helped propel the knowledge of other plants from around the world and this has helped accelerate the development of new supplements and medicines. In terms of the volume of medicinal plants exported, India ranks second in the world. There is thus an enormous scope for India to emerge as a major player in the global herb based medicines and products by developing its Research and Development capability.

PUBLIC AND PRIVATE SECTOR RESEARCH AND DEVELOPMENT CAPABILITY: BOOSTING QUALITY OF PLANT DERIVED MEDICINES

Although India has not been able to convert the grey areas of Ayurveda into a major contributor to National Economy, it has in particular, huge opportunities for the development of the pharmaceutical and phyto-chemical industry. The pharmaceutical industry is both large and successful. During the last two decades, it has made massive investments on pharmacological, clinical and chemical researches all over the world in an effort to discover still more potent plant drugs. About 250,000 living plant species contain a much greater diversity of bioactive compounds than any chemical library made by humans but only few plants species have been systematically investigated for the presence of bioactive compounds. A few new drug plants have successfully passed the tests of commercial screening.

The support for agricultural studies for commercial cultivation is needed to reap the benefits of this labour. In fact, agricultural studies on medicinal plants, by its very nature, demand an equally large investment and higher priority. Research in support of industrial development encompasses all activities ranging from the development of superior propagation materials, agro-technology, low cost and efficient processing technologies to improve quality and yield, new formulations to new products and the marketing of finished products. There has been capability building in India in recent years in the Research and Development sector of medicinal formulations involving plants and its compounds both in private sector (i.e. industry oriented) as well as government funded research. There are several private sector and government Research and Development institutions in India of which a few have been mentioned.

Among the industry oriented Research and Development institutes are Dabur Research Foundation, Himalaya Health Care, Zandu pharmaceuticals, Avestha Gengraine Technologies, Reliance Life Sciences, Hamdard, etc.

Dabur Research Foundation carries out research in diverse areas like ayurvedic research which relates traditional knowledge with modern science, pharmaceutical research, phyto-pharmaceuticals, biotechnology, personal care products, new drug and peptide research, food research, clinical research, etc.

The mission of Himalaya Health Care is to satisfy each customer's health needs through well researched, effective and safe remedies harnessed from nature's wealth. The company (claims to be completely research-oriented,) believes that the ideal healthcare system lies in the synergy between ayurveda and modern science.

Zandu's Research and Development activities are dedicated towards, highlighting the usefulness of Ayurvedic herbo-mineral products by scientific studies and promote and establish natural products beyond India, meeting export obligations.

Avestha Gengraine Technologies has chalked out a plan to address global functional food market in the form of 'food for medicine' under which a major thrust is to derive novel molecule and therapeutics from standardized plant extracts and prepare new cocktails for specific disease like diabetics and skin care.

Reliance Life Sciences is an emerging company focusing

Table 2. Global market for herbal supplements in 1999 (Raskin et al. 2002).

Region	Herbal supplements (Billion US $)
World	> 15
Europe	7.0
North America	3.0
Japan	2.4
Rest of Asia	2.7

on selected species for research to enhance both the quality and quantities of products of secondary metabolites (like pharmaceuticals, antibodies, anticancer agents, immune-modulators, flavour and fragrances) using Metabolic Engineering.

Apart from these there are several government established Research and Development institutions. Central Institute of Medicinal and Aromatic Plants (CIMAP) is committed to provide global standards for plant based research, processes and products using green technology mode to ensure sustained clientele from agriculture, society and industry. The National Botanical Research Institute (NBRI) has been undertaking both basic and applied research in various aspects of plant sciences for the conservation and sustainable utilization of plant genetic resources for human welfare and sustainable development. One of the major Research and Development activities at Central Drugs Research Institute (CDRI) is the exploration of terrestrial plants, including Indian traditional remedies for novel molecules for drug development. Several Regional Research Laboratories (RRL) are also involved in the regional MAP conservation and proper utilization through Research and Development. RRL-Thiruvananthapuram is involved in search for bioactive/polymer compounds from natural resources and development of new synthetic systems of technological interest; agro-processing of and value addition to spices, coconut, oil palm, cassava, etc. Laboratories at the Indian Institute of Chemical Biology, and the School of Natural Product Studies, Jadavpur University both in Kolkata, Indian Agriculture Research Institute etc. are making major contributions in the field of herbal research. The government of India has set up several Centres through the Indian System of Medicine and Homeopathy of which the National Medicinal Plant Board (NMPB) is one of them. The NMPB was set up to coordinate matters relating to medicinal plants, including developing policies and strategies for conservation, proper harvesting, cost-effective cultivation, Research and Development, processing, and marketing of raw materials to protect, sustain and develop the sector. There are several other agencies both in private sector and of under the government, that are involved in research and development activities of MAPs, along with conservation and upbringing of new plant-based products in national and international markets.

CONSERVING MAPs FOR DRUG/ HEALTH CARE DEVELOPMENT

The global craving for more herbal ingredients creates possibilities for the local cultivation of medicinal crops as well as for the regulated and sustainable harvest of wild. The expanding trade in medicinal plants has serious implications on the survival of several plants species, with many under serious threat to become extinct. According to an all India ethno biological survey carried out by the Ministry of Environment and Forests, Government of India, there are over 8000 species of plants being used by the people of India. 90-95% collection of Medicinal plants is from the forests (wild-collected). Few are cultivated. The biodiversity loss is not only a threat to ecology of the planet but also a more immediate threat to the livelihood security of rural communities. Data on threatened species are rare but national studies show 120 medicinal plants are rare or endangered in India. Open access to medicinal plants in the wild is perhaps one of the main reasons for the current unsustainable levels of harvesting. As the prices paid to the gatherers tend to be very low, commercial plant gatherers often 'mine' the natural resources rather than manage them, as their main objective is to generate an income resulting in destructive harvesting.

Other factors contributing to unsustainability include lack of sufficient data on wild plant populations, marketing, and trading; inadequate regulations and legal protection (including intellectual property rights for local practitioners with local knowledge); and poor access to appropriate technology for sound harvesting and plantation development. Interaction between social, economic and ecological systems is most essential to conserve the medicinal plants making its sustainable use. A need-based research including screening of plants for biological activity and focus on environmental and bio-diversity conservation aspects of forests, which continue to be primary habitats of medicinal plants, is desirable.

Training to the collectors and growers proves very useful in improving the quality of the material and plummeting the wastage. There is an urgency to have clearly defined

policies to regulate medicinal plant conservation, cultivation quality control standards, processing and preservation, marketing and trade including domestic and export, and a well-coordinated information network effort.

The modern pharmaceutical industry requires a large quantity of authentic plants too for manufacture of drugs. Opportunities should be given to industry and other beneficiaries to participate more directly in conservation and sustainable use of medicinal plants and their habitat. Reliance Life Sciences and few other industries claim to cultivate MAPs in suitable agroclimatic conditions. Both in situ and ex situ conservation of endangered plant species are in progress.

The National Medicinal Plants Board was set up by the Ministry of Health and Family Welfare, Government of India in 2000 to coordinate and implement policies relating to medicinal plants both at central and state level to facilitate inter ministry, inter state and institutional collaboration and to avoid duplication of efforts. The Forest Department of the Great Himalayan National Park, India, is promoting cultivation of medicinal plants as an income generating enterprise linked to conservation (Hamilton, 2003). Such efforts need to be replicated to speed up the conservation issue. The Foundation for Revitalisation of Local Health Traditions (FRLHT) is an NGO striving towards this issue and is quite successful in some parts of India especially in the south. There are many such foundations and institutions that are helping local people to conserve MAP for sustainable livelihood.

CONCLUDING REMARKS

Medicinal herbs as potential source of therapeutics aids has attained a significant role in health system all over the world for both humans and animals not only in the diseased condition but also as potential material for maintaining proper health. A major factor impeding the development of the medicinal plant based industries in developing countries has been the lack of information on the social and economic benefits that could be derived from the industrial utilization of medicinal plants. Except for the use of these plants for local health care needs, not much information has been available on their market potential and trading possibilities. As a result, the governments or entrepreneurs have not exploited the real potential of these plants (de Silva, 1997). About two million hectares of forest area on intensive management can produce medicinal plants for export and domestic use to provide health for our millions. Such effort will enhance greenery, generate employment and income to the people and conserve bio-diversity (Kumar, 2000). Further, a roadmap of cultivation of specific species (to start with few important ones) based on exploitable available resources and future demand is urgently required to facilitate a concrete policy coupled with incentive and plan of implementation.

India has the knowledge and skill to develop its Research and Development capabilities. It is the second largest exporter of medicinal plants. Instead of exporting such a large amount of valuable resource with very low returns it can think about developing its own Research and Development capabilities and produce finished goods in the form of modern medicines and health care products derived from plant origin and based on the knowledge of alternative system of medicine. Standardisation of products is most essential to compete in the world market that India has to lay stress on. The finger printing and marker compound analyses are nowadays gaining momentum for standardisation of traditional medicinal formulations. This technique not only helps in establishing the correct botanical identity but also helps in regulating the sanctity of the herb (Mukherjee, 2003). Accrediting body needs to be set up. Products have to be scientifically validated and a campaign to prove the safety of the products needs to be initiated. The Department of Indian System of Medicine and Homeopathy has been specially dealing with the rules and regulations for the herbals along with the Drugs and Cosmetic Act and has come up with the rules for the implementation of good manufacturing practices in herbals, which will not only help to make quality herbal products but also safeguard the adverse effects of the herbals (Mukherjee, 2002). With all these, India has to take up the challenge of leading the drug and herbal market while conserving its rich heritage through proper planning and implementation of policies.

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EXHIBIT 3

Reference source: Dictionary of Natural Products on DVD, Version 16:2, Dec. 2007, 210,000 natural products listed.

RCB_CT,197915Derivative: 7-O--D-Glucuronopyranoside
Synonym(s): **Baicalin**. Baicalein 7-glucuronoside. Baicaloside
Chapman Hall Number: KRL40-H
CAS Registry Number: 21967-41-9
Type of Compound Code(s): ZQ3700 XA3850 VK5030 ZQ0820
Molecular Formula: C21H18O11
Molecular Weight: 446.367
Accurate Mass: 446.084915
Percentage Composition: C 56.51%; H 4.06%; O 39.43%
Biological Source: Isol. from Scutellaria spp., Oroxylum indicum and other plant spp.
Biological Use/Importance: Diuretic
Melting Point: Mp 220-222
Optical Rotation: []28D 140
Solubility: Sol. MeOH, Et2O; poorly sol. H2O, hexane
Partition Coefficient (Calculated): Log P 1.62 (calc)

RCB_CT,156084Variant: (2R,3S)-form
Synonym(s): (+)-trans-form. **Catechin**. Catechol. Cianidanol, INN, JAN. Dexcyanidanol. Cianidol. Catergen. Drenoliver. Biocatechin. Tanningenic acid. Cyanidol. Gambircatechin. Catechuic acid. C.I. Natural Brown 3
Chapman Hall Number: CML34-P
CAS Registry Number: 154-23-4
Type of Compound Code(s): VK1100 XA2370 WA2800 WI2700 WI4600 WI3500 WI4000 ZQ5900
Molecular Formula: C15H14O6
Molecular Weight: 290.272
Accurate Mass: 290.07904
Percentage Composition: C 62.07%; H 4.86%; O 33.07%
Biological Source: Widespread in plants. First isol. in 1832 from Gambir-catechu (from Nauclea gambir) (most early isolates a mixt. of (+)-catechin and ()-epicatechin)
Biological Use/Importance: Possesses antiulcer props. Formerly used in the treatment of hepatic disorders
Physical Description: Cryst. + 4H2O (AcOH aq.)
Melting Point: Mp 93-96. Mp 175-177 (anhyd.)
Optical Rotation: []D +17
Partition Coefficient (Calculated): Log P 0.38 (uncertain value) (calc)
Other Data: Not to be confused with 1,2-Benzenediol HJX14-Z
Hazard Toxicity: Severe and occasionally fatal haemolytic anaemia reported when used therapeutically. LD50 (mus, ipr) 1000 mg/kg. Exp. reprod. and teratogenic effects
RTECS Accession Number: DJ3450000
Fluka: 22110

RCB_CT,197909Entry Name: 5,6,7-Trihydroxyflavone
Synonym(s): 5,6,7-Trihydroxy-2-phenyl-4H-1-benzopyran-4-one, 9CI. **Baicalein**. Noroxylin
9781572,263

Chapman Hall Number: HCW36-F
CAS Registry Number: 491-67-8
Type of Compound Code(s): ZQ3700 VK5030 XA1625 XA2400

Molecular Formula: C15H10O5
Molecular Weight: 270.241
Accurate Mass: 270.052825
Percentage Composition: C 66.67%; H 3.73%; O 29.60%
Biological Source: Isol. from Scutellaria spp. and other plants
Biological Use/Importance: Shows anti-HIV activity and antifungal activity
Physical Description: Yellow or brown-yellow prisms (MeOH or EtOH)
Melting Point: Mp 223-226. Mp 263-264 dec.
Solubility: Sol. MeOH, Et2O; poorly sol. H2O, hexane
Partition Coefficient (Calculated): Log P 1.05 (calc)
UV: [neutral] max 247 (58800); 274 (42669); 323 (17780) (MeOH) (Berdy)

Aldrich: 46511-9
Fluka: 11712
Sigma: B7277

EXHIBIT 4

Safety and efficacy of flavocoxid compared with naproxen in subjects with osteoarthritis of the knee: a pilot study

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ABSTRACT

Purpose: The present study was designed to compare the effectiveness and safety of flavocoxid, a dual pathway inhibitor anti-inflammatory agent of botanical origin, to naproxen in a population of subjects with moderate-severe OA.

Methods and Materials: In this double blind study 103 Russian subjects were randomly assigned to receive either flavocoxid (500 mg BID) or naproxen (500 mg BID) for one month. Outcome measures included the short WOMAC scale (validated in Russian) subject VAS scales for discomfort and global response, investigator VAS for global response and fecal occult blood.

Results: Both groups noted significant reduction in the signs and symptoms of knee OA. There were no statistically detectable differences between the flavocoxid and naproxen groups with respect to any of the outcome variables ($p \leq 0.001$) nor with respect to any adverse event although there was a trend toward a higher incidence of edema in the naproxen group.

Conclusions: In this short term pilot study flavocoxid appeared to be as effective as naproxen in controlling the signs and symptoms of OA of the knee. A low incidence of adverse events was reported for both groups.

Key words: Osteoarthritis, Flavocoxid, Flavonoids, Medical Foods, Dual Pathway Anti-Inflammatory Agents.

MeSH: Osteoarthritis [C05.550.114.606]; Anti-Inflammatory Agents [D27.505.954.158]; Flavonoids [D03.438.150.266.450]

BACKGROUND

Osteoarthritis (OA) is the most common form of joint disease in adults worldwide affecting more than 40 million people in the United States alone.[1] In addition to physical therapy, analgesics and intra-articular injections of corticosteroids or hyaluronate preparations, NSAIDs and COX-2 inhibitors are the mainstay of chemical therapies. While effective at relieving pain and inflammation, their use is often limited by toxic effects on the gastrointestinal tract, kidneys, platelets, heart and liver. These adverse effects are mediated by molecules generated via the primary enzyme pathways involved in arachidonic acid metabolism, cyclooxygenase-1 and -2 and 5-lipoxygenase (**Figure 1**) all of which serve important physiologic functions.[2,3] It is thought that imbalance in the levels of these end products from selective blocking of one or another metabolic pathway may account for much of the toxicity of anti-inflammatory agents.[4,5]

Flavocoxid is a proprietary medical food product for the dietary management of the metabolic processes involved in the pathogenesis of OA. In pre-clinical

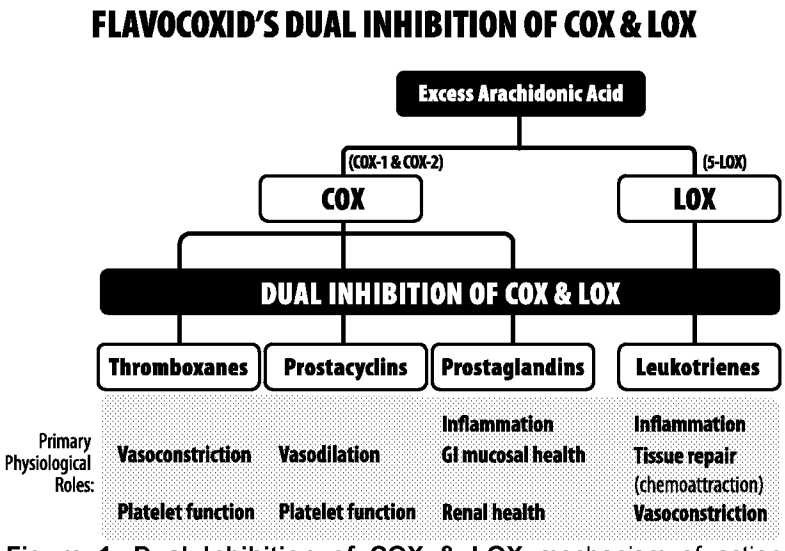

Figure 1. Dual Inhibition of COX & LOX mechanism of action regulates production of prostaglandins and leukotrienes to manage joint inflammation, without over-inhibition of either that is necessary for GI health and other physiological functions. [6]

biochemical assays the product has been shown to possess significant action against the primary enzyme pathways involved in arachidonic acid metabolism, cyclooxygenase-1 and 2 and 5-lipoxygenase.[6] When compared with other anti-inflammatory agents, flavocoxid is a more balanced inhibitor of all major pathways. Dual pathway inhibition greatly reduces the downstream production of inflammatory mediators and results in an improved toxicity profile.[7] Prior studies have suggested that flavocoxid may have a beneficial effect in the management of OA. This study is designed to compare the efficacy and safety of flavocoxid compared to naproxen in subjects with moderate-severe OA of the knee and to provide guidance for the development of future studies.

Table 1. Baseline Characteristics

	Flavocoxid 500 mg BID	Naproxen 500 mg BID
N per group	52	51
Age: Mean (sd)	60.3 (9.7)	57.5 (14.4)
Sex: (N male; N female)	7 M; 45 F	6 M; 45 F
Weight (KG): Mean (sd)	82.3 (14.1)	83.0 (15.8)
BMI: Mean (sd)	30.4 (6.3)	30.7 (5.1)
WOMAC (composite score): Mean (sd)	55.3 (17.0)	55.8 (15.1)
PGAD (mm on VAS): Mean (sd)	57.1 (16.7)	57.7 (14.2)
SGAD (mm on VAS): Mean (sd)	33.2 (16.1)	36.3 (15.2)
SGADc (mm on VAS): Mean (sd)	56.8 (19.4)	58.2 (18.2)

Table 1. Baseline characteristics of subjects in both flavocoxid and naproxen groups were similar. WOMAC = Western Ontario and McMaster's University (WOMAC) Osteoarthritis; PGAD = Physician's Global Assessment of Disease; SGAD = Subject's Global Assessment of Disease; SGADc = Subject's Global Assessment of Discomfort.

PRODUCT

Under U.S. FDA law, flavocoxid is classified as a medical food, a category distinct from drugs and supplements. Flavocoxid is a blend of B-ring flavonoids and flavans extracted from two botanical sources, Scutellaria baicalensis and Acacia catechu, concentrated and standardized to approximately 98% purity. In Asia, these compounds have been used for more than 1000 years for treatment of a variety of inflammatory conditions. Recently, this action has been shown to be related to inhibition of COX-1, COX-2 and 5-LOX, the major pathways of arachidonic acid metabolism in joints. Thus, flavocoxid is the first so-called dual pathway inhibitor to reach market.

PURPOSE

This study was designed to compare the effectiveness and safety of full therapeutic doses of flavocoxid and naproxen in subjects with osteoarthritis (OA) of the knee using conventional efficacy and safety endpoint parameters.

STUDY DESIGN

This was a 4 week, multi-center, double-blind, active comparator controlled study performed in the Russian Republic. Subjects were chosen from investigators' hospital clinic practices and were required to have Kellgren-Lawrence grade 2-3 osteoarthritis of a knee in need of anti-inflammatory therapy. Subjects were required to discontinue taking NSAIDs (including selective COX-2 inhibitors) at least 2 weeks prior to the screening visit. Acetaminophen was provided for rescue analgesia.

Efficacy parameters included subject VAS for discomfort and global disease activity, investigator global assessment of disease activity and short WOMAC (validated in Russian). The short form WOMAC has been validated as a surrogate for the full WOMAC.[8]

Major inclusion criteria are:
1. Grade 2-3 K-L OA in at least one knee
2. Age 35 to 85, inclusive
3. In general good health
4. Not pregnant or breast feeding

Major exclusion criteria are:
1. Grade 1 or 4 OA in target knee
2. Grade 4 OA in any knee or hip
3. Any form of arthropathy other than OA
4. Any musculoskeletal or neurologic condition that might alter gait or confound evaluation of discomfort in the target knee
5. Use of NSAIDs (including selective COX-2) inhibitors within 2 weeks of the screening visit
6. Use of any gastroprotective medication whether by prescription or OTC within 2 weeks of the screening visit
7. Intra-articular corticosteroids within 3 months or hyaluronate preparations within 6 months of the screening visit

Table 2. Improvements in WOMAC and VAS

	Flavocoxid 500 mg BID (N = 52)		Naproxen 500 mg BID (N = 51)			
	Patients with Improvement N (%)	Improvement Mean (sd)	Patients with Improvement N (%)	Improvement Mean (sd)	Within-group p-value	Between-group p-value
WOMAC	41 (79%)	32% (33)	45 (88%)	36% (36)	<.001	.67
PGAD	43 (83%)	31% (32)	38 (75%)	38% (35)	<.001	.34
SGAD	45 (87%)	162% (249)	45 (88%)	130% (188)	<.001	.46
SGADc	45 (87%)	28% (39)	45 (88%)	38% (39)	≤.001	.20

Table 2. Fisher's Exact Test: Over 75% of both flavocoxid and naproxen groups showed improvement. Within group improvements were statistically significant. Differences between groups were not statistically significant. WOMAC = Western Ontario and McMaster's University (WOMAC) Osteoarthritis; PGAD = Physician's Global Assessment of Disease; SGAD = Subject's Global Assessment of Disease; SGADc = Subject's Global Assessment of Discomfort.

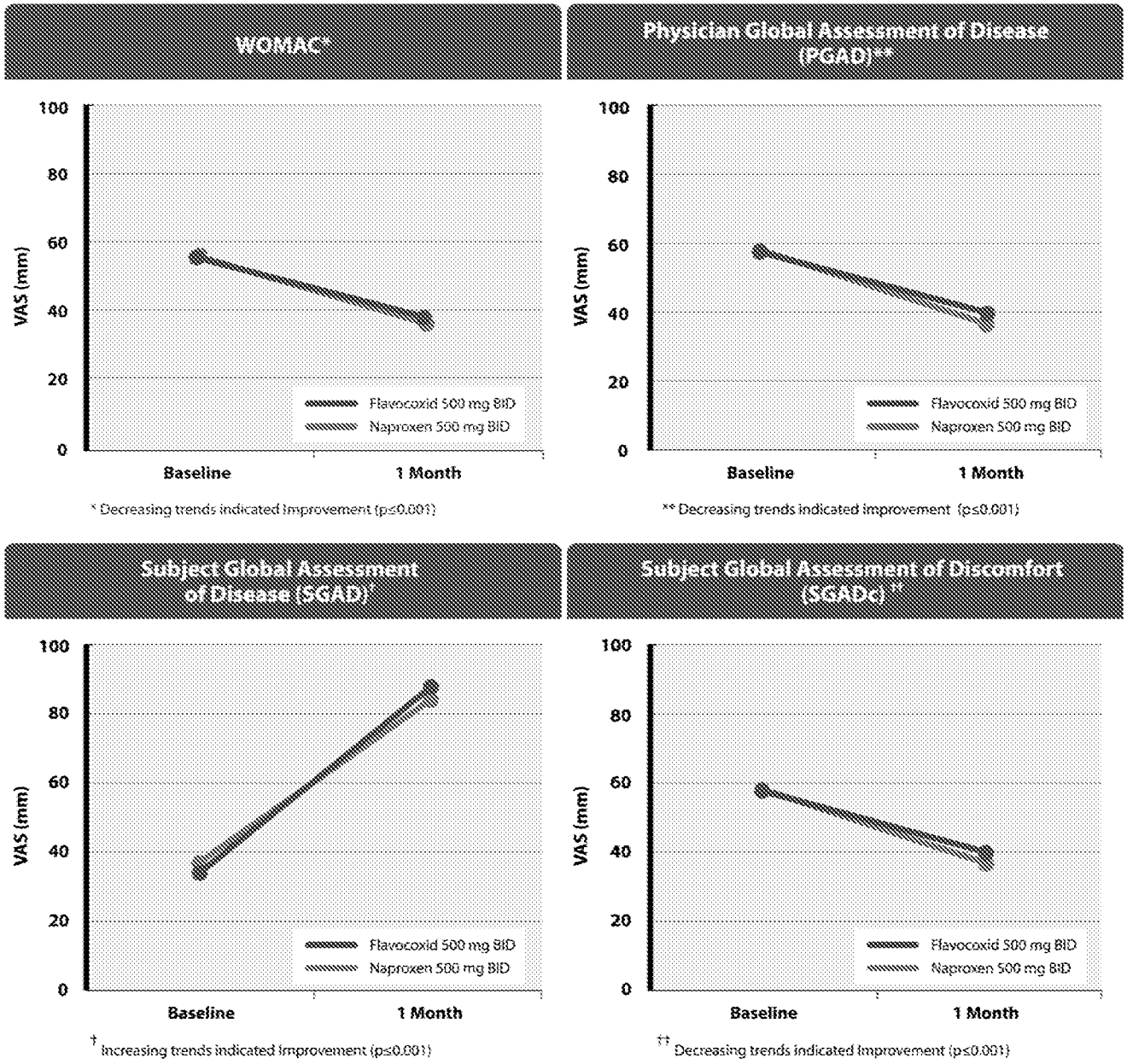

Figure 2. Efficacy endpoints. Within group improvements were all significant for both flavocoxid and naproxen groups (p≤0.001). Differences between groups were not statistically significant (p>0.2).

8. Use of mechanical ambulation aids
9. History of bleeding disorder or use of anticoagulant medications
10. History of chronic upper gastrointestinal disease or upper GI bleeding within 3 years of screening
11. Positive fecal occult at screening
12. Significant renal, cardiovascular or neoplastic disease or any other disease that, in the opinion of the investigator might put the subject at undue risk during the study
13. History of allergy to aspirin, NSAIDs or flavonoids
14. Current substance abuse including consumption of >1 unit of alcohol daily
15. Participation in another clinical trial within 30 days or 7 half lives of the prior study product, whichever is longer

RESULTS

One hundred three (103) subjects were randomized to the trial. Two (2) subjects, both in the naproxen arm, failed to complete the full month of study product, one for increasing upper GI discomfort, the other for personal reasons unrelated to the trial. Both, however, completed three (3) weeks of therapy and were included in the efficacy and safety analyses. The baseline characteristics of the two groups are shown in **Table 1**. There were no differences in demography or in WOMAC or VAS scores between the two groups.

Fisher's exact test was computed for Improved vs. Not Improved (sum of unchanged and worsened) for all parameters (**Table 2**). Both groups improved significantly in all parameters (75% – 88%) showing within group p values ≤0.001. No significant differences were found between groups for any of the four efficacy parameters measured, although there was a slight trend toward greater improvement in PGAD in the flavocoxid group and WOMAC in the naproxen group.

Both flavocoxid and naproxen groups improved significantly on all efficacy primary endpoints (p≤0.001 within groups), as shown in **Figure 2**. The flavocoxid and naproxen groups performed nearly identically, and the between group differences were not statistically significant for all efficacy endpoints.

The overall adverse event (AE) rate was about the same for both groups, 46% for flavocoxid group and 51% for naproxen group (**Table 3**). Neither the numbers nor kinds of AEs differed statistically between the groups, with the exception of a slight but not statistically significant trend toward more frequent edema and nonspecific musculoskeletal events in the naproxen group. No significant changes were observed within or between groups for weight, systolic blood pressure, or diastolic blood pressure. No positive fecal occult bloods were recorded.

Table 3. Adverse Events

	Flavocoxid 500 mg BID (N = 52)	Naproxen 500 mg BID (N = 51)
Cardiovascular	**4 (8%)**	**6 (12%)**
Worsening Hypertension	2	2
Edema	2	4
Dermal (itching)	**1 (2%)**	**0 (0%)**
Dizziness/Insomnia	**1 (2%)**	**1 (2%)**
Vertigo	1	0
Insomnia	0	1
Gastrointestinal	**11 (21%)**	**11 (22%)**
Abdominal pain	3	4
Constipation	0	1
Diarrhea	3	2
Heartburn	2	1
Nausea	1	3
Vomiting	2	0
Musculoskeletal (Increased knee pain)	**3 (6%)**	**6 (12%)**
Respiratory (Cold, URI)	**3 (6%)**	**0 (0%)**
Other (Facial swelling)	**1 (2%)**	**2 (4%)**
TOTAL	**24 (46%)**	**26 (51%)**

DISCUSSION

Flavocoxid is a proprietary blend of botanical extracts containing primarily baicalin and catechin, concentrated and standardized to approximately 98% purity. These compounds have been extracted from plants that have been used medicinally as anti-inflammatory agents in Asia for more than 1000 years.

In the present study, flavocoxid was shown to be as effective as naproxen in controlling the signs and symptoms of moderate-severe knee OA. Response rates are consistent with those reported in many other NSAID efficacy studies in OA, although the SGAD may be somewhat exaggerated compared to that seen in American and European trials. The length of the study was probably too short to demonstrate differences in safety between the two products. The complete absence of positive fecal occult blood tests is consistent with this hypothesis.

The data support the need to perform additional clinical studies to further evaluate both the safety and

efficacy of flavocoxid in larger populations over longer time courses.

CONCLUSION

Flavocoxid and naproxen appear to be equally effective therapies for symptomatic osteoarthritis of the knee. Except for a trend toward increased edema and nonspecific musculoskeletal discomfort in the naproxen group, flavocoxid and naproxen appear to be equally safe when administered in full therapeutic doses for the short term of this study.

ACKNOWLEDGEMENT

The sponsor, Primus Pharmaceuticals Inc, Scottsdale Arizona, USA and the authors, wish to thank AmeRuss, a contract research organization based in Scottsdale, Arizona for their competent and timely organization and management of this study.

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